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Lymphocyte and Plasma Vitamin C Levels in Type 2 Diabetic Patients With and Without Diabetes Complications

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Diabetes has been considered to be associated with oxidative stress. It has been suggested that increased free radicals and decline of antioxidant defense mechanisms induce diabetic micro- and macrovascular complications (1–3). Vitamin C is one of the major antioxidants and is detected in various blood components (4). However, measurements of vitamin C levels have shown inconsistent results, and the interpretation of vitamin C levels in diabetes as an antioxidant biomarker has not been clarified (5–8). In this study, we investigated the lymphocyte and plasma vitamin C levels in type 2 diabetic patients with and without diabetes complications.

RESEARCH DESIGN AND METHODS— Forty-one patients with type 2 diabetes (63 ± 8.9 years [mean ± SD]; 25 men and 16 women) attending the Department of Endocrinology and Metabolism at Shizuoka City Hospital were recruited. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria. The duration of illness was 11 ± 8.3 years, fasting plasma glucose was 137 ± 43 mg/dl, and HbA_{1c} levels were 7.1 ± 1.0%. Twenty-six patients had diabetes complications with neuropathy, retinopathy, or nephropathy, and 15 patients had no complications. Both diabetic groups were matched by age, sex, fasting plasma glucose, and HbA_{1c} level (63 ± 9.7 years, 18 men and 8 women, 137 ± 45 mg/dl, and 7.2 ± 1.0% for diabetic patients with complications compared with 64 ± 7.5 years, 7 men and 8 women, 137 ± 42 mg/dl, and 6.8 ± 0.8% for diabetic patients without complications, respectively). The duration of illness was longer in the diabetic patients with complications than in diabetic patients without complications (13 ± 9.1 vs. 7.7 ± 5.2 years, respectively, $P = 0.051$). For the normal control subjects, 50 age- and sex-matched healthy volunteers (63 ± 5.7 years, 31 men and 19 women) were recruited. The participants taking vitamin supplements were excluded from the study. All participants gave informed consent before entering the study. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee at the hospital.

Blood samples were obtained by vein puncture in the morning while the patients were in the fasting state. Lymphocytes and plasma were prepared by centrifugation and the Ficoll gradients method, then immediately treated with metaphosphoric acid (final 5% wt/wt) to stabilize vitamin C (9,10). These processes were performed within 2 h under cooled conditions on ice to obtain reliable data. The vitamin C samples were stored at −80°C until analyzed, and the vitamin C (ascorbic acid, reduced form) levels were measured by high-performance liquid chromatography with the electrochemical detector method (11). All samples were handled and stored similarly in both diabetic patients and control subjects.

The lymphocyte and plasma vitamin C levels in type 2 diabetic patients were compared with those of the control subjects. The differences between the vitamin C levels in type 2 diabetic patients with and without diabetes complications were also studied. Statistical analysis was performed with the unpaired Student's *t* test to compare the data between diabetic patients and control subjects and between type 2 diabetic patients with and without diabetes complications. A P value <0.05 was considered significant.

RESULTS— The lymphocyte vitamin C level in diabetic patients was significantly lower than in control subjects (18 ± 4.5 vs. 28 ± 7.9 nmol/mg protein, $P < 0.0001$), whereas the plasma vitamin C level was not different (59 ± 19 vs. 53 ± 18 μmol/l, $P = 0.17$) (Fig. 1*A* and *B*). There were no significant linear correlations between the lymphocyte and plasma vitamin C levels in diabetic patients ($r = 0.011$, $P = 0.95$) as well as in control subjects ($r = 0.14$, $P = 0.35$). The lymphocyte vitamin C level in diabetic patients with complications was significantly lower than in those without complications (17 ± 3.3 vs. 21 ± 5.4 nmol/mg protein, $P = 0.011$) (Fig. 1*C*), whereas the plasma vitamin C level was not different (59 ± 18 vs. 59 ± 21 μmol/l, $P = 0.97$).

CONCLUSIONS— Increased oxidative stress in diabetes could contribute to depletion of antioxidants such as vitamin C (2,3). In this report, we demonstrated that the lymphocyte vitamin C level is significantly lower in type 2 diabetic patients, but we could not observe such an association in plasma vitamin C levels. The plasma concentration of vitamin C is considered to be strongly correlated with transient consumption of foods such as fruit, supplements, and vegetables (4).

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

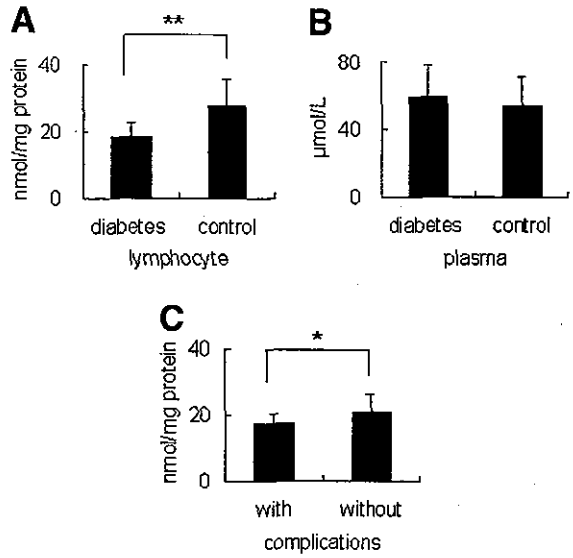

Figure 1—*Lymphocyte and plasma vitamin C levels in type 2 diabetic patients (n = 41) and control subjects (n = 50). A: Lymphocyte vitamin C level in diabetic patients was significantly lower than that in the control subjects (**P < 0.0001). B: Plasma vitamin C level in diabetic patients was not different from that in the control subjects (P = 0.17). C: Lymphocyte vitamin C level in diabetic patients with complications (n = 26) was significantly lower than that in those without complications (n = 15) (*P = 0.011). The horizontal bars represent the mean ± SD.*

Compared with plasma, lymphocyte has been reported to maintain a vitamin C concentration as large as 80- to 100-fold across the plasma membrane (12,13) and to have cell-membrane transporting mechanisms between vitamin C and glucose (14,15). In diabetes, therefore, the measurement of lymphocyte vitamin C might be expected to be a more reliable antioxidant biomarker than plasma vitamin C level.

It is unclear whether leukocyte vitamin C correlates with diabetes complications. VanderJagt et al. (5) reported that vitamin C levels in mononuclear leukocytes were decreased in the whole group of type 1 diabetic patients compared with control subjects but were not different between patients with and without long-term complications. We showed the significant lower lymphocyte vitamin C levels in patients with type 2 diabetes with complications compared with those without complications. However, the results should be interpreted carefully because of the small sample size and because the differences of lymphocyte vitamin C level among different diabetes complications are not fully clarified. Further studies are required to investigate the precise correlations of lymphocyte vitamin C with duration or severity of diabetes and to establish the clinical usefulness of lymphocyte vitamin C level as a biomarker in developing diabetes complications.

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オボアルブミン誘発食物アレルギー発症に対する高ビタミンE食投与の影響

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はじめに

近年、我が国では食物アレルギー、アトピー性皮膚炎、花粉症などのアレルギー疾患を持つ人が著明に増加しており問題となっている。その原因として、高蛋白・高栄養な食生活や、大気汚染、花粉、ダニなどのアレルゲンの増加、社会的ストレスの増加など多くの要因の関与が考えられている。最近、ヒトおよび実験動物において高ビタミンE(VE)摂取がアレルギーの発症、進展に対して有益な効果をもたらすことが見出され、その効果が期待されている[1)-3)]。本研究では、オボアルブミン(OVA)誘発食物アレルギーモデルマウスを用いてアレルギー発症に対する高VE食投与の影響について検討した。また、これまでの報告のほとんどはVEとしてα-トコフェロール(α-Toc)を用いているが、今回の実験ではα-Tocに加えてγ-トコトリエノール(γ-T3)の抗アレルギー作用についても併せて検討を行った。

方法

実験動物としてBALB/cマウス、雌、8週齢を用いた。1週間予備飼育後、Halterenら[4)]の方法により実験開始時にOVA 2μgと水酸化アルミニウムゲル(ALUM)25μlを腹腔内投与し、さらに14日目にOVA 1μgを腹腔内投与後、18日目にOVA 1 mgを経口投与することにより食物アレルギーモデルマウスを作成した(図1)。

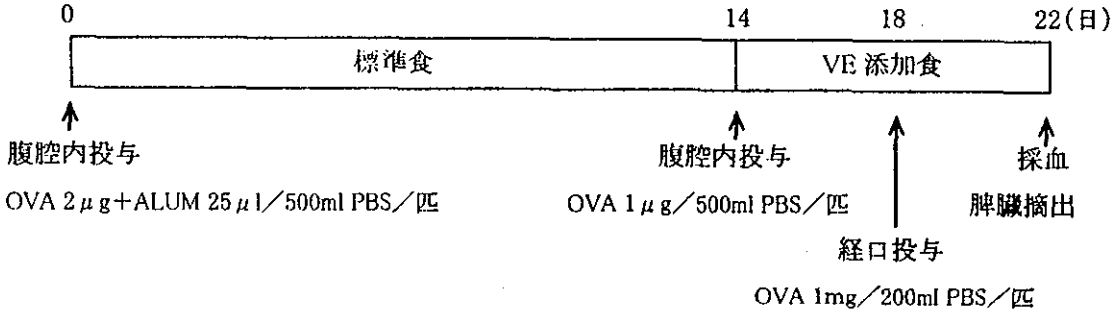

図1 食物アレルギーモデルマウスの作成

実験期間中、コントロール群は標準食(50mg α-Toc/kg)にて飼育した。VE 添加食群は 2 回目の感作までは標準食を与え、その後から標準食にα-Toc またはγ-T3 を 200 または 450 mg/kg 添加した食餌を与えた。実験期間中の摂食量および体重については週 1 回測定し、OVA 経口投与の 4 日後の 22 日目に採血し、脾臓を摘出した。採取した血液から血清を分離し、血清総 IgE および OVA 特異的 IgE 濃度を ELISA 法により測定した。免疫能として、Con A、PHA 等のマイトジェンおよび OVA 刺激による脾臓リンパ球幼若化能、ヘルパーT(CD4)およびサプレッサーT(CD8)細胞割合、Con A 刺激48時間後の脾臓リンパ球培養上清中のインターロイキン4(IL-4)濃度について検討した。

結果

1. 体重、脾臓重量ならびに脾臓リンパ球数

実験期間中の摂食量については、α-Toc およびγ-T3 投与の影響はみられず、コントロール群と各 VE 添加食群との間に差異を認めなかった。体重、脾臓重量および脾臓リンパ球数についても、コントロール群と各 VE 添加食群との間に有意な差異を認めなかった(表 1)。

表 1 体重、脾臓重量ならびに脾臓リンパ球数

Groups	Body wt. (g)	Spleen wt. (g/100g BW)	Splenic lymphocytes no. (10^{-7}/0.1g spleen)
Control	23.5±0.6	0.46±0.03	3.44±0.36
200mg/kg α-Toc	23.3±0.2	0.43±0.03	3.47±0.40
450mg/kg α-Toc	24.5±0.5	0.41±0.01	3.63±0.13
200mg/kg γ-T3	24.3±1.2	0.36±0.03	2.44±0.36
450mg/kg γ-T3	24.3±0.5	0.41±0.02	3.05±0.44

2. 血清総 IgE および OVA 特異的 IgE 濃度

血清総 IgE 濃度は、コントロール群と比較し 450 mg/kg α-Toc 添加食群において低い傾向を認めたものの、γ-T3 添加食群については変化を認めなかった(図 2)。

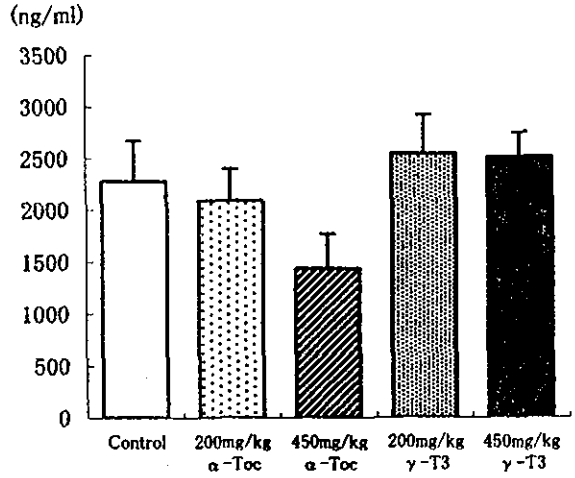

図 2 血清総 IgE 濃度

血清 OVA 特異的 IgE 濃度は、コントロール群と比較し 200 mg/kg α-Toc 添加食群においては低い傾向を認め、さらに 450 mg/kg α-Toc 添加食群においては有意な低下を認めた。また、450 mg/kg γ-T3 添加食群の血清 OVA 特異的 IgE 濃度は、コントロール群と比較してやや低い傾向を認めた(図 3)。

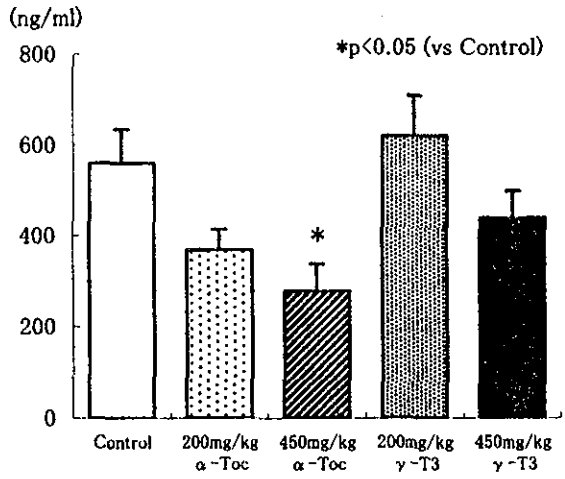

図 3 血清 OVA 特異的 IgE 濃度

3. 脾臓リンパ球幼若化能

Con A および PWM 刺激に対する脾臓リンパ球幼若化能は、コントロール群と比較し各 VE 添加食群において高い傾向を認めた。しかし、PHA および LPS 刺激に対する脾臓リンパ球幼若化能は、コントロール群と比較し 450 mg/kg α-Toc 添加食群において逆に低い傾向を認めた。一方、OVA 刺激に対する脾臓リンパ球幼若化能は、コントロール群と比較し 450 mg/kg α-Toc 添加食群において有意に高いことを認めた(図 4)。

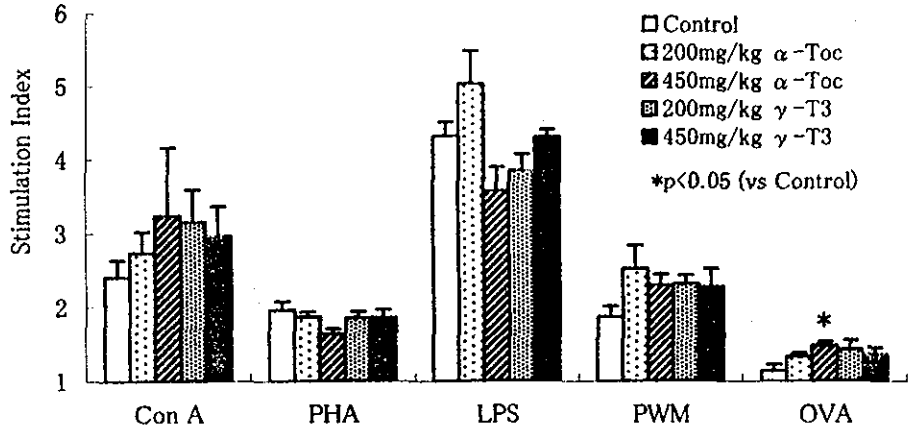

図 4 脾臓リンパ球幼若化能

4. ヘルパーT(CD4)およびサプレッサーT(CD8)細胞割合、CD4/CD8 比

ヘルパーT(CD4)細胞割合は、コントロール群と比較し 450 mg/kg α-Toc 添加食群において低い傾向を認めた。サプレッサーT(CD8)細胞割合については、コントロール群と各 VE 添加食群との間に有意な差異を認めなかった(図 5)。また、CD4/CD8比については、コントロール群と比較し 450 mg/kg α-Toc 添加食群においてやや低い傾向を認めた(図 6)。

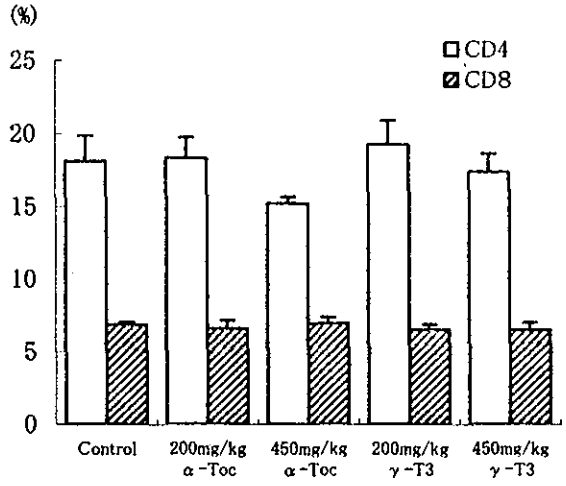

図 5 細胞割合

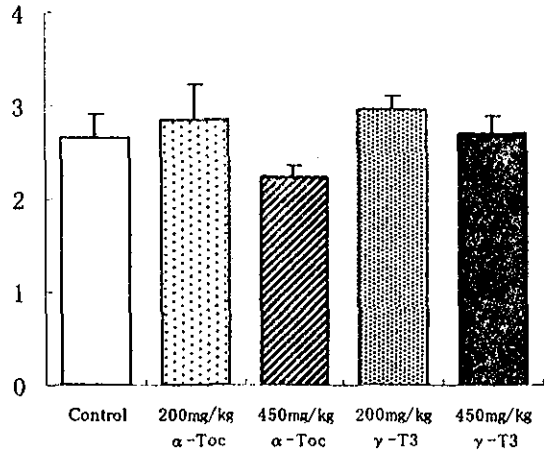

図 6 CD4／CD8 比

5. Con A 刺激に伴う脾臓リンパ球からの IL-4 産生

Con A 刺激に伴う脾臓リンパ球からの IL-4 産生は、コントロール群とα-Toc 添加食群との間に有意な差異を認めなかった。しかし、γ-T3 添加食群では IL-4 産生がコントロール群と比較し高い傾向を認めた(図7)。

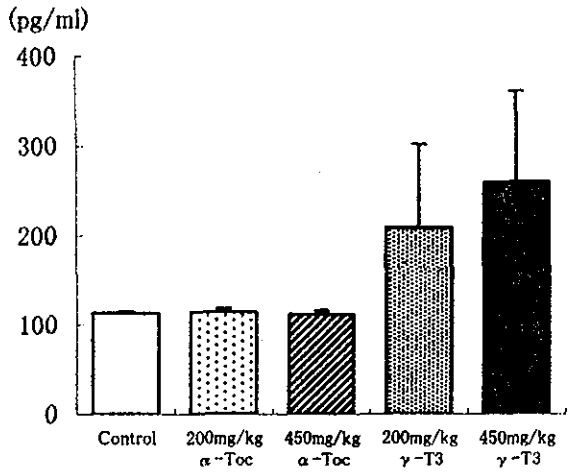

図 7 Con A 刺激に伴う脾臓リンパ球からの IL-4 産生

考察

本研究では、OVA 誘発食物アレルギーモデルマウスを用いて食物アレルギー発症に対する高 VE 食投与の影響について検討した。

その結果、食物アレルギー発症に伴い高値を示す血清総 IgE および OVA 特異的 IgE 濃度の上昇が、高 α-Toc 食摂取により抑制されることを認めた。このことは、鼻アレルギーモデルマウスを用いた Zheng らの知見[1]と一致する。また、今回は γ-T3 についても検討を行ったが、明らかな IgE 産生の抑制を認めなかった。図 8 に要約したように、IgE 産生に至るまでにはマクロファージ(MΦ)、ヘルパーT(Th)細胞、B 細胞ならびに形質細胞の関与が知られている。今回の研究では、α-Toc 添加食群において PHA および LPS 刺激に対する脾臓リンパ球幼若化能および Th 細胞割合の低下を認めた。このことから、高 α-Toc 食摂取により Th 細胞および B 細胞機能が低くなっているために IgE 産生が抑制されたものと考えられる。また、脾臓リンパ球からの IL-4 産生については、α-Toc の影響を認めなかったことから、IgE 産生の抑制とは関連しないことが示唆される。VE のアレルギー抑制機序として、α-Toc が直接的に IgE の産生を抑制すること[5]や犬の肥満細胞腫において *in vitro* の α-Toc 添加によりヒスタミンおよびプロスタグランジン D_2(PGD_2)の放出が抑制されること[6]などが報告されている。今後さらにマスト細胞からのヒスタミン遊離など VE による抗アレルギー作用の詳細なメカニズムを解明していく必要がある。

以上、本研究により、高 α-Toc 食摂取により OVA 誘発食物アレルギー発症に伴う IgE 産生が抑制されることを認め、そのことが Th 細胞および B 細胞幼若化能および Th 細胞割合の低下と関連することが示唆された。

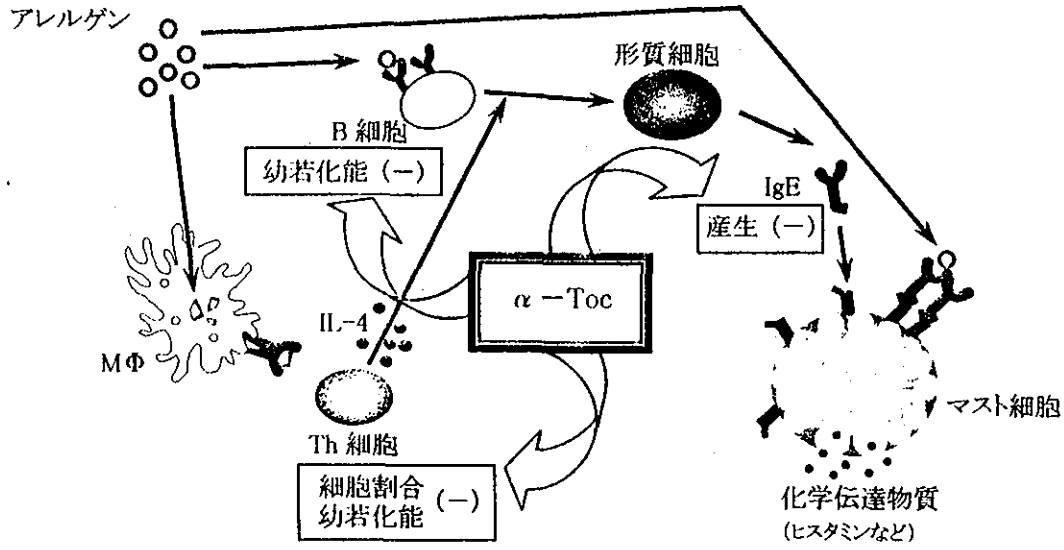

図 8 α-トコフェロールによる食物アレルギーの発症抑制メカニズム

[文 献]

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5

Nutrients to Stimulate Cellular Immunity: Role in Cancer Prevention and Therapy

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Introduction

Nutrition plays an important role in two respects: one is the inhibitory factor for carcinogenesis via its immunoenhancing effects and the other is the determinant for prognosis of cancer patients. In this chapter, the relationship between the malnourished status of cancer patients and immune responses, and the beneficial effect of nutritional support using enteral and parenteral feeding are described first. The rest of this chapter discusses the immunomodulating action of each nutrient, such as fat, protein (amino acid), vitamins, and minerals, in cancer patients or animals with tumors. Finally, some foods that promote health are described in regard to their action on host immunity.

Nutritional Status in Cancer Patients and Immunity

Not only cancer itself but also conventional approaches to therapy for cancer such as chemotherapy, operative therapy, and radiation therapy are known to produce profound changes in host immunity. The effects of chemotherapy upon immune responses are related both to the dosage and duration of therapy and are readily reversible. Operative therapy likewise suppresses both humoral and cell-mediated immunity for 2 to 3 weeks, as manifested by in vitro and in vivo tests of these functions. And radiation therapy induces the decrease of host immune responses for more prolonged periods of time over 10 years. One of the factors inducing the decrease of host immunity following cancer and its therapies is malnutrition. Nutritional status affects both limbs of the immune system. Enteral and parenteral (intravenous hyperalimentation) nutrition are safe and effective methods for correcting deficits in cancer patients. In the malnourished gastric cancer patient, one week of pre- or postoperative parenteral nutrition significantly increased natural killer cell (NK) activity, T-helper proportion, T-helper/T-suppressor ratio, and total T lymphocyte count (Yan 1990). This evidence suggests that perioperative nutrition support can improve the immunocompetence of gastric cancer patients. The other study has shown that a marked depression of NK activity of peripheral blood mononuclear cells (PBMC) was observed in malnourished cancer patients with moderate protein-calories malnutrition, but

not in well-nourished cancer patients nor in the healthy controls (Villa et al. 1991). Although the decreased NK activity in this study was restored to normal by rIL-2, but not by α-rIFN, the ability to produce IL-2 in vitro in each cancer patient did not correlate with NK activity. This evidence suggests that malnutrition, rather than malignancy, plays a major role in the immune dysfunction of cancer patients. When compared with immune functions after operation in patients with esophageal or gastric cancer, ConA- and PHA-stimulated lymphocyte proliferation decreased significantly 7 days after esophagectomy, but was unchanged in the patients receiving gastrectomy (Tashiro et al. 1999). Since serum cortisol level was significantly increased in patients after surgery, stress response may induce in part the suppression of immune functions. In patients with hepatocellular carcinoma, the phagocytic and bactericidal activities of neutrophils and the percentage of NK cells were significantly reduced (Iida et al. 1999). In particular, the phagocytic and bactericidal activities of neutrophils were low in patients with poor nutritional status compared to those with a good nutritional status. Taken together, nutritional supplementation such as enteral and parenteral nutrition for malnourished cancer patients appears to be useful for preventing further decrease of host immune functions.

It is a well-known fact that food restriction results in longer longevity than ad libitum feeding (Sheldon et al. 1995). Some previous animal studies have found that food restriction has a beneficial effect on the incidence of cancer. Using mice treated with 3-methylcholanthrene (MC), 40% dietary restriction caused a great inhibition of tumor incidence at 114 days after treatment(Konno et al. 1991). Since the optimum duration and degree of dietary restriction cause the enhancements of both splenic lymphocyte proliferation and phagocytic activity of alveolar macrophages in rats (Fig. 5.1), the decreased incidence of cancer may be related to the changes of host immune functions following dietary restriction. In fact, the above study has shown that dietary restriction causes a marked increase of the proportion of Thy1.2+, L3T4+T cells, and increased T cell responses against ConA and IL-2 in MC-treated diet-restricted mice. The increase of host immune functions might be one of the major causes for the reduction of tumor occurrence by dietary restriction. In conclusion, caution should be employed in the nutritional manipulation of malnourished cancer patients.

Role of Lipids in Cancer Prevention and Immunity

There is in vitro and in vivo evidence to suggest that dietary lipids play an important role in modulating immune functions. It is known that diets high in polyunsaturated fat, relative to diets high in saturated fat, are more immunosuppressive and are better promoters of tumorigenesis (Vitale and Broitman 1981). It has been also shown that rats fed diets high in lipid and cholesterol develop more 1,2-dimethylhydrazine (DMH)-induced bowel tumors than those fed diets low in lipid or without cholesterol. When rats were fed diets containing 20% safflower or coconut oil, with or without cholesterol (1%) and cholic acid (0.3%), for 35 weeks and concomitantly given DMH, the suppression of PHA response was observed in the polyunsaturated fat (safflower oil) diet group compared with the saturated fat (coconut oil) diet group (Kraus et al. 1987). And the addition of cholesterol to

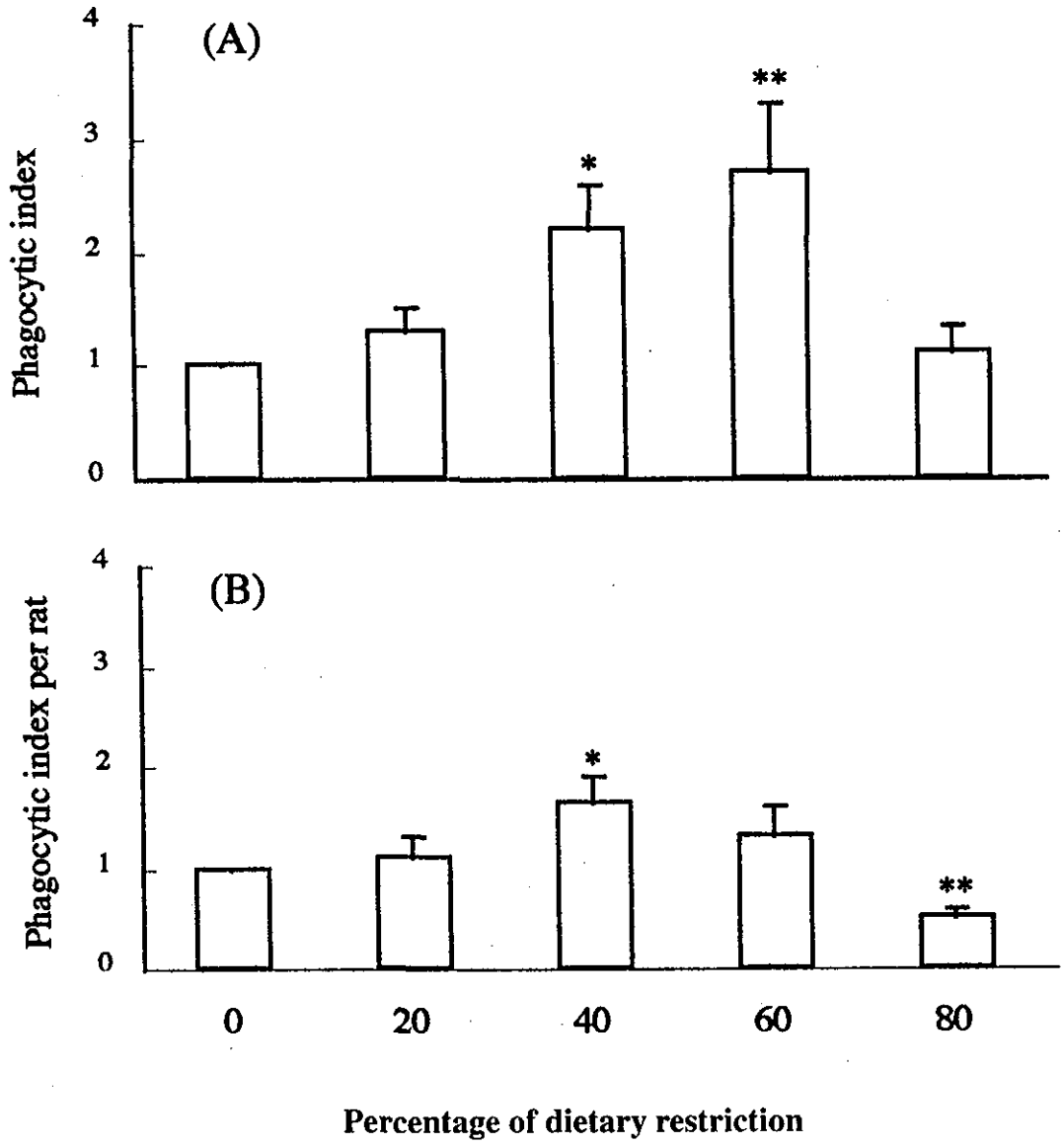

Figure 5.1. Phagocytic activity of alveolar macrophages (AM) (2 × 105) in rats (A) or AM per rat (B) fed mildly or moderately restricted diets (20 to 80% restriction to control) for 2 weeks. Phagocytic index was calculated by assigning 1 to phagocytic activity of control group and comparing this to phagocytic activity of other groups. Values are means ± SD of triplicate cultures; significantly different from controls (*P < 0.05, **P < 0.001). (Reproduced from J. Nutr. Sci. Vitaminol., 35; Moriguchi, S., Toba, M. and Kishino, Y., Effects of dietary restriction on cellular immunity in rats, 49–59. Copyright 1989, with permission from the Center for Academic Publications Japan)

either the polyunsaturated or saturated fat diet diminished PHA response, to a lesser degree, of T lymphocytes from rats fed these diets. However, natural killer (NK) cell activity was unaffected by either the difference of dietary fat or cholesterol. The other study has shown that the splenic lymphocyte transformation response induced by ConA, PHA, or pokeweed mitogen is significantly depressed in the rats fed 24% corn oil (vehicle-treat-

ed) and in the DMH-treated rats fed 5% fat compared with the vehicle-treated rats fed 5% fat (Locniskar et al. 1986). This study has also found that splenic NK cell cytotoxic activity was not significantly affected by dietary fat, DMH treatment, or tumor development. On the other hand, it has been found that corn oil administered by oral gavage retards mononuclear cell leukemia proliferation, which is mediated at least in part by enhancing immune competence (Hursting et al. 1994).

As described previously in this chapter, the beneficial effect of early postoperative enteral nutrition enriched with not only arginine and RNA but also omega-3 fatty acids was found in 78 patients undergoing curative operations for gastric or pancreatic cancer (Braga et al. 1996). Since prealbumin concentration, retinol-binding protein (RBP) concentration, delayed hypersensitivity responses, phagocytic ability of monocytes, and concentration of interleukin-2 (IL-2) receptors had recovered more in the patients receiving the enriched enteral solution, early enteral feeding is likely to induce the recovery of both their nutritional and immunological status quicker than those supported with standard enteral diet or total parenteral nutrition (TPN). The recent study has also shown that the supplementation of eicosapentaenoic acid (EPA) with soybean oil emulsion significantly improved the lymphocyte proliferation and natural killer cell activity compared with the group receiving only soybean oil emulsion (Furukawa et al. 1999). Furthermore, the other study was conducted to investigate the effect of immunological effects of three TPN regimens such as calories derived solely from glucose and a half of total calories derived from lipid emulsion (one as long-chain triglycerides and the other containing half the fat as long-chain triglycerides and a half as medium-chain triglycerides) in patients undergoing preoperative parenteral nutrition. This study has shown that NK activity and lymphokine-activated killer (LAK) activity were significantly higher after TPN with long-chain and middle-chain triglyceride solutions and a significant fall in LAK activity occurred after TPN with long-chain triglyceride solution (Fig. 5.2) (Sedman et al. 1991).The design of TPN regimens is also an important factor for cancer patients to improve or maintain their immune functions.

Role of Protein or Amino Acids in Cancer Patients and Immunity

As described above, nutritional status is the most important determinant in the prognosis for cancer patients. It is well accepted that protein-calorie malnutrition impairs host immunity with particular detrimental effects on the T-cell system, resulting in increased opportunistic infection and increased morbidity and mortality in hospitalized patients including cancer patients (Daly et al. 1990). Levels of vitamins A and E, having a potent enhancing effect on host immune functions and being low in tumor bearing animals, decreased further when maintained in the restricted diet without soybean, but were raised to normal following addition of soybean in the diet (Mukhopadhyay et al. 1994). As soybean protein has high arginine content, the enhancing effect of soybean on immune functions in animals fed the restricted diet may be in part due to arginine. In fact, there are many reports showing that arginine has an immunoenhancing effects and an inhibitory

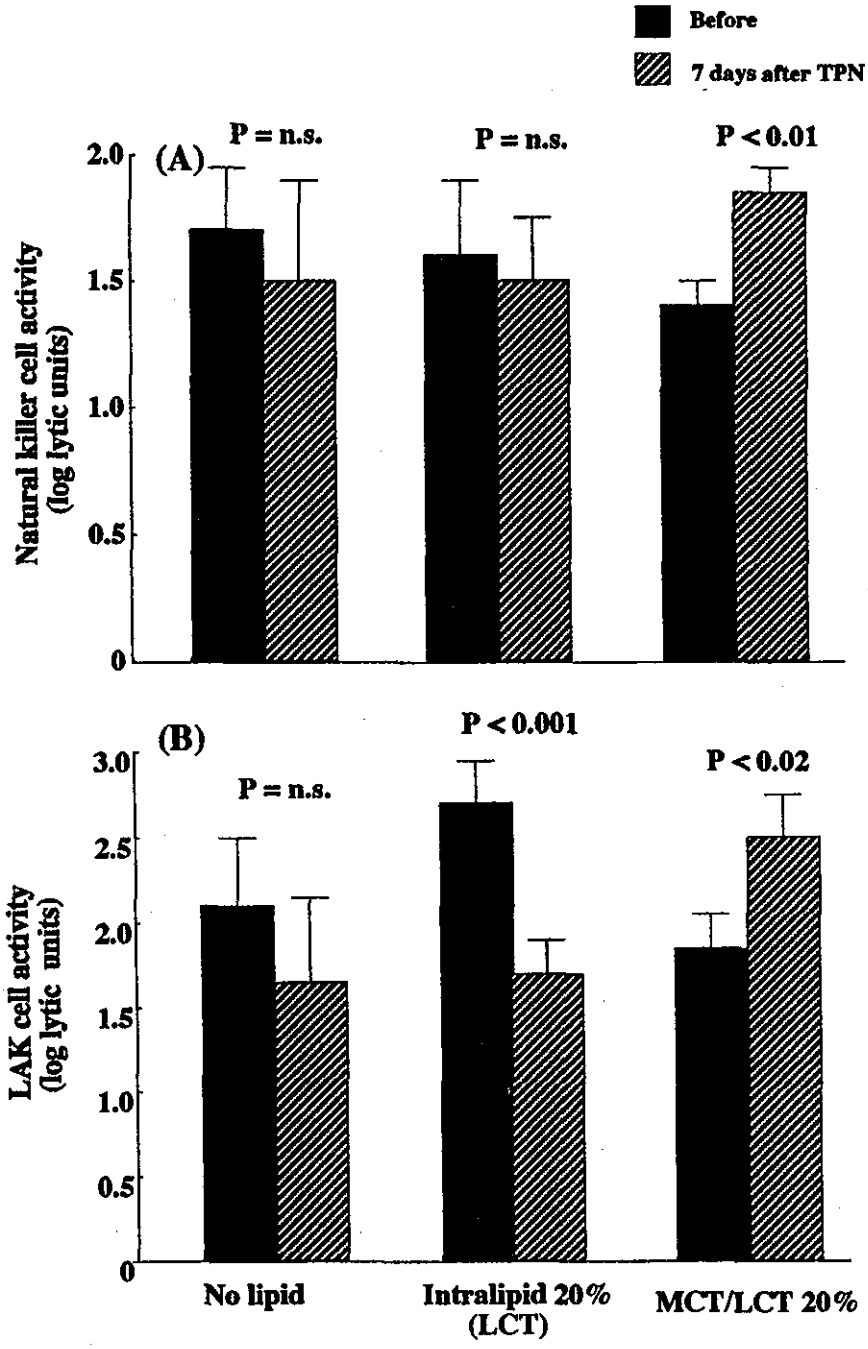

Figure 5.2. Changes in spontaneous natural killer cell activity (A) and the capacity to induce lymphokine-activated killer (LAK) cells in response to interleukin-2 (B) before and 7 days after total parenteral nutrition (TPN) with each of the three TPN regimens. Histograms denote means ± SEM of log-transformed data. MCT/LCT , medium-chain triglycerides/long-chain triglycerides. (Reproduced from Br. J. Sur, 78; Sedman, P. C., Somers, S. S., Ramsden, C. W., Brennan, T. G., and Guillou, P. J., Effects of different lipid emulsions on lymphocyte functions during total parenteral nutrition, 1396–1399. Copyright 1991, with permission from Butterworth-Heinemann Ltd.)

effect on tumor growth and metastasis. In vitro incubation with arginine induced threefold increase of NK cell activity of human PBL and 1.5-fold increase of human monocyte-mediated cytotoxicity (Fig. 5.3) (Moriguchi et al. 1987). Production of tumor cytotoxic factor from human monocytes also significantly increased after in vitro incubation with arginine. This evidence suggests that arginine action against tumor cells is due to not only the enhancement of host immune functions such as NK activity and human monocyte cytotoxicity but also to increased production of cytokines having the direct effect on tumor cells. Using arginine-enriched amino acids solution, growth and metastases of Yoshida sarcoma were suppressed (Tachibana et al. 1985). Since arginine supplementation enhanced the phagocytic activity of rat alveolar macrophages, the authors concluded that

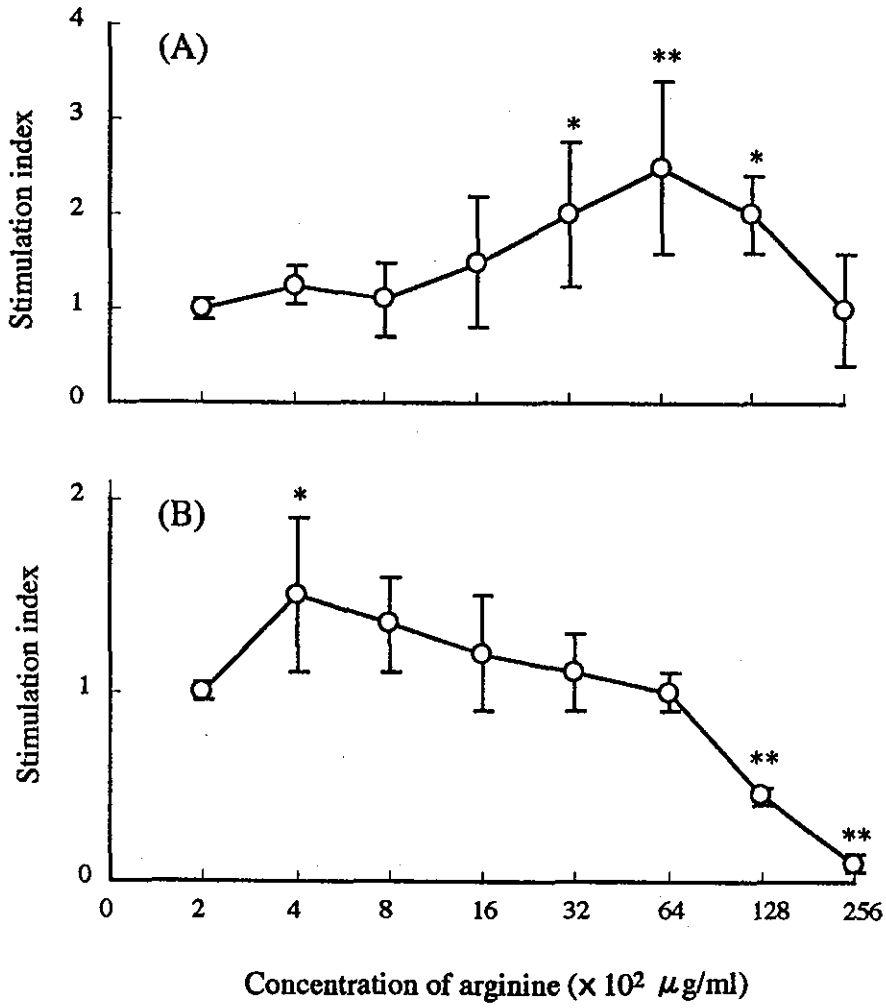

Figure 5.3. Natural killer (NK) cell activity (A) and monocyte cytotoxicity (B) after incubation with various concentrations of arginine for 48 hours. Bars are means ± SD of triplicate cultures and compared with that of control culture (2×10^2 μg/ml of arginine). NK activity and percent cytotoxicity of monocytes in control culture are 22.0 ± 1.6% and 11.0 ± 0.8 %, respectively; significantly different from control culture (*$P < 0.05$, **$P < 0.01$). (Reproduced from Nutr. Res., 7; Moriguchi, S., Mukai, K., Hiraoka, I., and Kishino, Y., Functional changes in human lymphocytes and monocytes after in vitro incubation with arginine, 719–729. Copyright 1987, with permission from Elsevier Science)

the suppressive effect of arginine-enriched solution on tumor growth and metastases may be due to its activation of the immunologic system. Some of the clinical studies and animal studies were designed to evaluate the effect of arginine plus other nutrients such as glutamine, RNA, omega-3 fatty acid, and ornithine 2-oxoglutarate on host immune response (Gianotti et al. 1999, Chuntrasakul et al. 1998, Kemen et al. 1995).

Supplemented diet or enteral diet has a beneficial effect on host immune functions. Recently, it has been found that arginine is a substrate for nitric oxide showing various physiological activities such as the regulation of arterial smooth muscle, blood pressure, and immune functions (Palmer et al. 1987, Haynes et al. 1993, Ding et al. 1988). On the other hand, it has been reported that the expression of adhesion molecule CD44 is closely associated with the degree of metastasis of tumors (Sikorska et al. 2002, Lakshmi et al. 1997). In fact, the higher metastatic B16 melanoma cells showed the higher expression of CD44 as shown in Figure 5.4 (Moriguchi et al. 2002). In addition, the expression of CD44 was significantly suppressed following in vitro incubation with SIN-1, spontaneously generating NO (Fig. 5.5), which resulted in the decreased lung metastases of B16 melanoma in mice fed the high arginine diet. These results suggest that arginine has an inhibitory effect on tumor growth and metastases via two different mechanisms, such as immunoenhancement and depressed expression of adhesion molecule CD44.

The prolonged use of total parenteral nutrition provokes mucosal atrophy of the small intestine (Grant and Snyder 1988), which is related to the lack of glutamine in standard currently available parenteral solutions. Because glutamine is poorly soluble and instable, it has been not generally used as one of the amino acids in parenteral nutrition. However, glutamine is a nutrient necessary for the intestinal mucosal metabolism as a major oxidative fuel. Alanylglutamine, glutamine-containing dipeptide, was found as a source of free glutamine in parenteral nutrition (Furst et al. 1989). On the other hand, although free glutamine is highly consumed by rapidly proliferating tumor cells, it was not clearly known whether tumor growth rate was increased by intravenous supplementation of alanylglutamine. In addition, it is known that glutamine is preferentially used for the provision of fuels in proliferating lymphocytes (Ardawi and Newsgolme 1982) and macrophages (Newsholme and Newsholme 1989). A study was undertaken and evaluated the changes of tumor volume and weight, and cellular immune response following the administration of an alanylglutamine-enriched solution. As a result, in vivo administration of alanylglutamine did not accelerate the growth of transplanted Yoshida sarcoma cells as measured by changes in the weight and volume (Kweon et al. 1991). And the addition of alanylglutamine to culture medium showed a significant increase in phagocytic activity of alveolar macrophages and in blastogenic response of splenocytes. These results suggest that alanylglutamine infusion does not stimulate tumor growth due to maintenance of some immunoenhancing effects by glutamine liberated from alanylglutamine in tumor-bearing hosts.

Role of Vitamins in Cancer Prevention and Immunity

Vitamin A is a nutrient having the most impact on both tumor incidence and growth and host immune system. Continuous administration of vitamin A and its derivatives

(retinoids) has been shown to prevent cancer of the skin (Verma et al. 1982), lung (Saffiotti et al. 1967), bladder (Moon et al. 1982), and breast (Moon et al. 1983) in experimental animals exposed to carcinogens. Epidemiological results also suggest that dietary retinoids may be chemopreventive to some forms of cancer in humans as well (Wald et al.

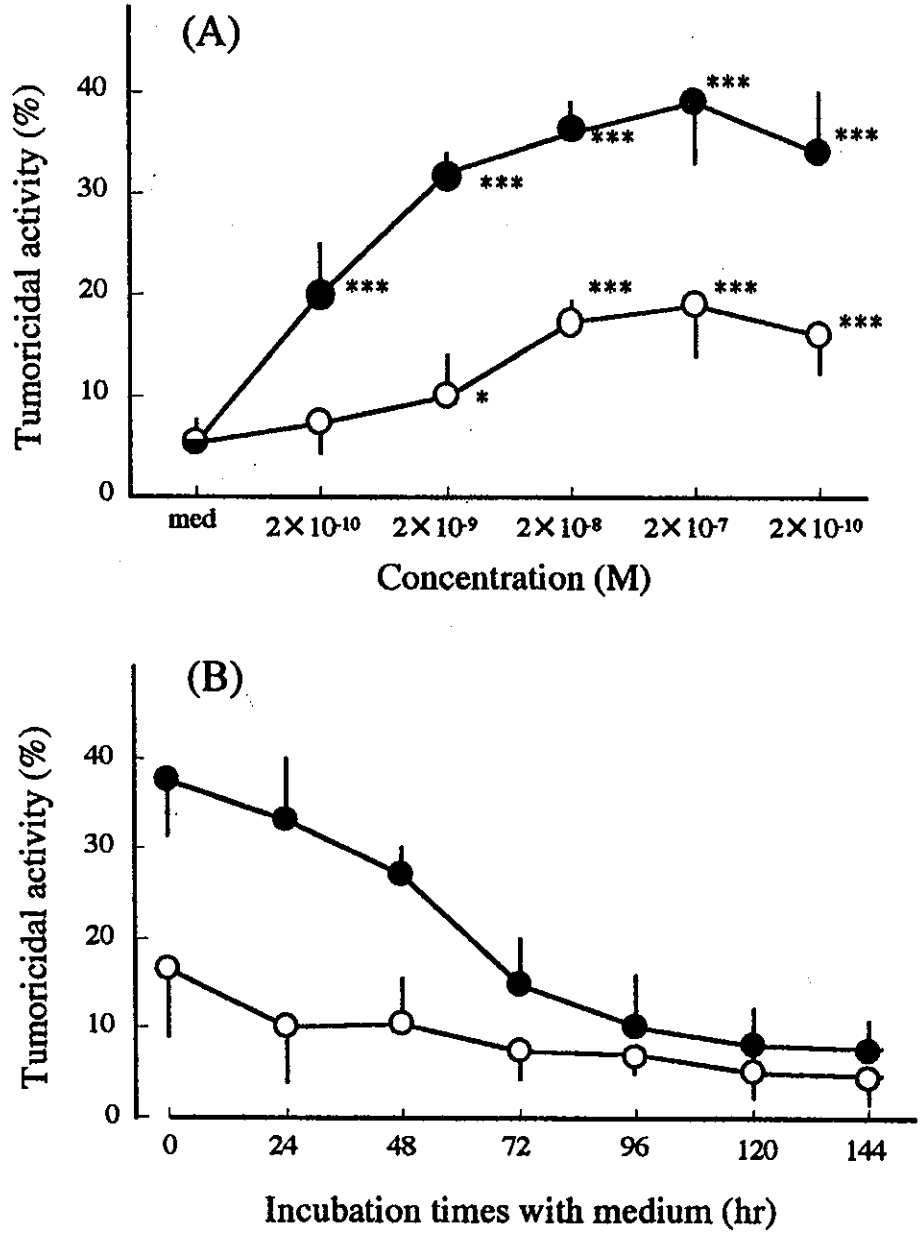

Figure 5.4. Tumoricidal activity of human monocytes treated in vitro with various concentrations (2×10^{-10} to 2×10^{-6} M) of beta-carotene or beta-carotene encapsulated in liposomes for 24 hours (**A**) and maintenance of the tumoricidal state of human monocytes after in vitro incubation with 2×10^{-7} M of beta-carotene or beta-carotene encapsulated in liposomes (**B**). Values are means ± SD for triplicate cultures; significantly different from cultures with medium containing 0.2% ethanol (*$P < 0.05$, ***$P < 0.001$). (Reproduced from Nutr. Res., 10; Moriguchi, S., and Kishino, Y ., In vitro activation of tumoricidal properties of human monocytes by beta-carotene encapsulated in liposomes, 837–846. Copyright 1990, with permission from Elsevier Science)

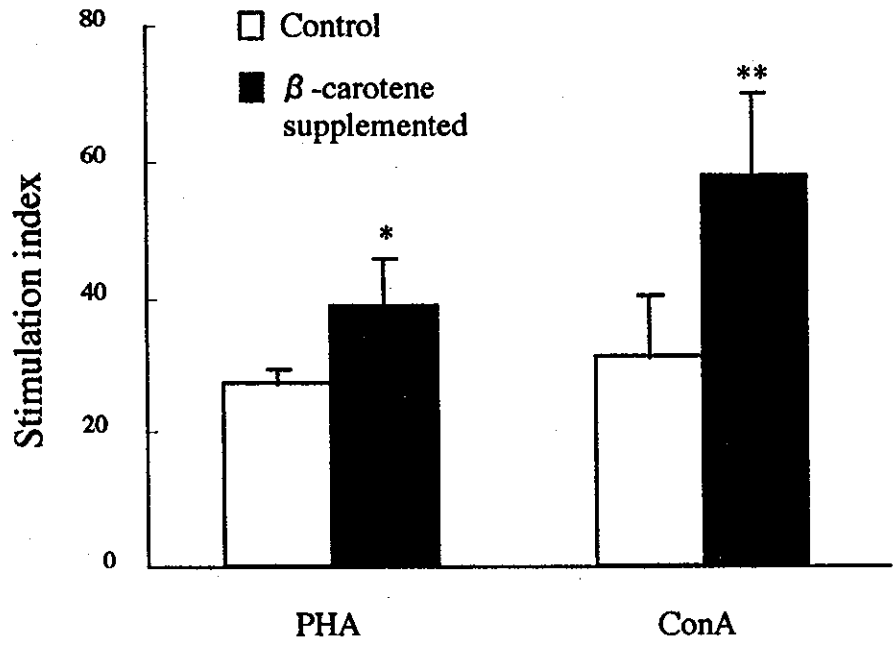

Figure 5.5. Proliferation of peripheral blood lymphocytes with PHA or ConA in control and beta-carotene supplemented subjects. Values are means ± SD; significantly different from control subjects (*P < 0.05, **P < 0.01). (Reproduced from Nutr. Res., 16; Moriguchi, S., Okishima, N., Sumida, S., Okamura, K., Doi, T., and Kishino, Y., Beta-carotene supplementation enhances lymphocyte proliferation with mitogens in human peripheral blood lymphocytes, 211–218. Copyright 1996, with permission from Elsevier Science)

1980, Kark et al. 1981). However, the toxicity of vitamin A precludes its use as a form of cancer prevention. The development of new vitamin A derivatives having low toxicity and high chemopreventive activity is required. The effect of selected doses of dietary retinyl palmitate and 13-*cis*-retinoic acid has been measured by using mouse skin papilloma promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Dietary retinyl palmitate yields a dose-dependent inhibition of the number and weight of tumors, whereas dietary 13-*cis*-retinoic acid resulted in a decrease of weight but not in number of tumors (Gensler et al. 1987). This result suggests that retinyl palmitate inhibits both incidence and growth of tumors, whereas 13-*cis*-retinoid acid inhibits not the incidence of tumors but tumor growth. Using the same dietary regimens, the high retinyl palmitate diets significantly increased phagocytic ability and tumoricidal activity of peritoneal macrophages and mitogenesis of splenocytes and thymocytes in mice (Moriguchi et al. 1985). This evidence suggests that high retinyl palmitate diets may cause activation of both peritoneal macrophages and lymphocytes. Furthermore, in nude mice, defecting thymus gland development and lacking functional mature T lymphocytes, retinyl palmitate diets significantly stimulated phagocytosis of peritoneal macrophages only at the highest level. T-cell-dependent mitogens did not also cause significant mitogenesis in any dietary group, while lipopolysaccharide (LPS), a B-cell mitogen, did (Watson and Moriguchi 1989). These results suggest that mature T cells may be needed for retinyl palmitate to produce normal activation of macrophages, except at very high retinyl palmitate levels. As the in vitro

study showed that both retinoids and carotenoids at higher concentrations have inhibitory effects on human lymphocyte functions, the use of vitamin A or its derivatives for chemoprevention and therapeutic trials for cancer patients should be carefully considered in its design.

Beta-carotene is one of the carotenoids, which are pigments contributing to the yellow, orange, and/or red coloration in vegetables and fruits. Epidemiological studies have demonstrated that a high intake of food rich in beta-carotene is associated with reduced risk of certain types of cancers, especially lung cancer (Le Marchand et al. 1989). Since other carotenoids lacking provitamin A activity had the similar anticancer effect as that of beta-carotene, it has been suggested that the anticancer effects of beta-carotene is not due to its provitamin A activity. Thus, the anticancer and antibacterial effects of beta-carotene are considered to be due to not provitamin A functions but antioxidant and immunomodulatory functions (Bendich and Shapiro 1986). Proliferation of peripheral blood lymphocytes with PHA or ConA was 1.4- to 1.9-fold higher in the beta-carotene supplemented group compared to the control group, whereas there was no significant difference in NK cell activity between both groups (Fig. 5.6) (Moriguchi et al. 1996). In addition, the study on in vitro effect of beta-carotene (beta) and beta-carotene encapsulated in liposomes (L + beta) on tumoricidal activity of human monocytes has found that the use of liposomes with beta-carotene could induce higher tumoricidal activity of human monocytes following short-term incubation and incubation with lower concentration compared to those of beta-carotene (Fig. 5.7) (Moriguchi and Kishino 1990). Since many other reports support the action of beta-carotene against inhibition of tumorigenesis and tumor cell growth (Kune et al. 1989), and the enhancement of immune responses (Bendich 1989), it is believed that beta-carotene is a nutrient for improving cancer patients and aged people showing decreased cellular immunity. Other fat-soluble vitamins, D and E, are also known to have both inhibitory effects on tumor incidence and growth (Mehta and Mehta 2002, Yu et al. 2002) and enhancing effects on host immune response (Lemire 2000, Moriguchi and Muraga 2000).

Water-soluble vitamins B_6 and C are also known to have the immunoenhancing effects. In the experiment using athymic nude mice, vitamin B_6 supplementation caused increased response of B lymphocytes with lipopolysaccharide (LPS), but did not inhibit the development of human malignant melanoma (M21-HPB) xenografts (Gebhard et al. 1990). This evidence suggests that tumor inhibition by high dietary vitamin B_6 may be mediated by T-lymphocyte-dependent mechanisms. Vitamin C is also an essential nutrient playing a role in protecting against carcinogenesis. As one of the inhibitory actions of vitamin C against carcinogenesis, the enhancement of cellular immunity is involved (Glatthaar et al. 1986). However, when desiring stable immunoenhancement, a daily high-level intake of vitamin C (> 1000 mg/day) is needed (Anderson et al. 1980).

Role of Minerals in Cancer Prevention and Immunity

Selenium (Se) is an essential nutritional factor with a chemopreventive potential. An inverse correlation between cancer incidence and dietary intake of Se has been well estab-

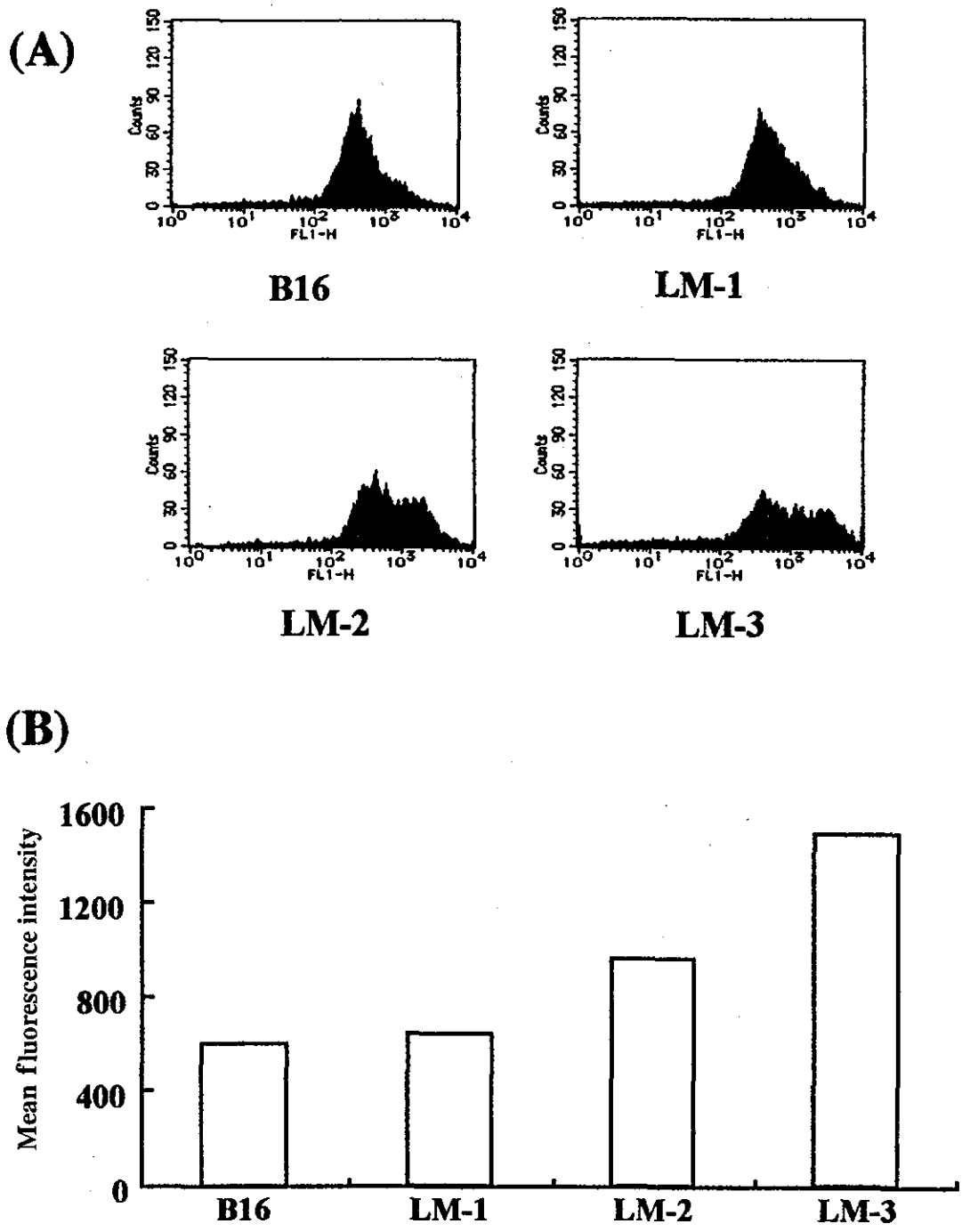

Figure 5.6. Expression of adhesion molecule, CD44 in B16 melanoma with various degrees of lung metastatic ability in male C57B1/6 mice. B16 melanoma cells with various degrees of lung metastatic ability were isolated from the lungs of mice. B16 melanoma cell lines in order of frequency of lung metastasis are LM-3, LM-2, LM-1, and B16. Distribution of melanoma cells with different fluorescence intensity (A) and mean fluorescence intensity in each melanoma cell line (B) are indicated. As shown in both figures, LM-3 cells having the high ability of lung metastasis showed the highest expression of CD44.